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PRINCIPAL INVESTIGATOR: Joshua L. Fischer, B.A.

CONTRACTING ORGANIZATION: Indiana University School of Medicine
Indianapolis, Indiana 46202

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Joshua L. Fischer, B.A.

E-Mail: jlfische@iupui.edu

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INTRODUCTION.

The goal of the research is to evaluate the contribution of DNA repair to the protective effects of selenium in the context of chemotherapy. Specifically, the aims are to determine whether selenium-induced protection from DNA-damaging chemotherapeutics *in vivo* corresponds to reduced myelosuppression. Second, determine if selenium-induced protection from chemotherapeutic toxicity corresponds to elevated DNA repair *in vivo*.

BODY.

Much of the preliminary work from the grant proposal has been published (Fischer et. al.). The carboplatin chemotherapy protocol was tested on wildtype mice. Mice were given 60 mg/kg carboplatin once per week i.p. Toxicity was evaluated by complete blood counts five days after each dose of chemotherapy. After 3 doses of 60 mg/kg of carboplatin the dose was escalated to 100 mg/kg to induce more significant myelosuppression. After 2 doses of 100 mg/kg the chemotherapy regimen was stopped. Figure 1 illustrates the suppression of white blood cells and platelets during the chemotherapy cycle.

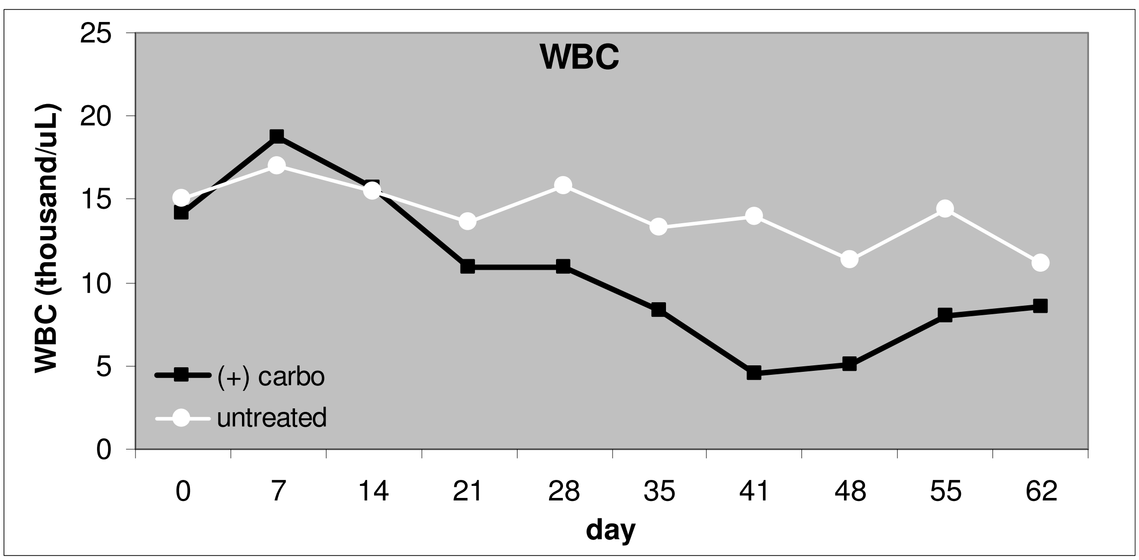

Figure 1. White blood cell counts from wildtype mice on a carboplatin chemotherapy regimen. Carboplatin treatment was administered at 60 mg/kg on days 9, 16, 23 and at 100 mg/kg on days 30 and 37. Figure is representative of 2 independent experiments.

Regarding the first task in the Statement of Work wildtype mice were given selenium every day for one week prior to starting the chemotherapy regimen. Rather than giving the selenium via oral gavage mice received supplemental selenium in the drinking water. The purpose of adding the selenium to the drinking water was to lessen the stress on the animals. Preliminary experiments indicated that there was some therapeutic benefit for the mice who received the selenium in the drinking water compared to the control mice.

Table 1 indicates parameters that were found to be significantly higher in the peripheral blood of the selenium treated mice.

Day	0	6	13	20	27	34	41
Total white blood cells			x				
Neutrophils			x				x
Lymphocytes			x				
Monocytes				x			x
Platelets						x	x
Hematocrit			x				x
Red blood cells			x				
Hemoglobin			x				x

Table 1. Analysis of complete blood counts from wt mice +/- selenomethionine during carboplatin treatment. "X" denotes a value that was significantly higher in the selenium-fed mice than the controls during the course of a chemotherapy cycle. Animals received 9 ppm selenomethionine in the drinking water for 7 days prior to starting the regimen and for the duration of the treatment cycle. Peripheral blood was collected once per week and a complete blood count was performed.

The results of the preliminary study indicate that selenium may exert some protective effect on the hematopoietic system. Following the preliminary experiment a separate study was initiated to determine the maximum tolerated dose of selenium in the drinking water. Mice were given increasing amounts of selenomethionine in the drinking water while being monitored for weight loss, indicating dehydration from not drinking the water. Mice tolerated 120 ppm selenomethionine in the drinking water without any weight loss. Table 2 shows the results of the study. It was decided that in order to better control the dose of selenium received by each mouse the selenomethionine should be administered via oral gavage as originally planned. An experiment using the oral gavage procedure has been initiated, but the results are not yet available.

Day	0	2	4	5	9	11	16	18	25
Weight	23.00	22.27	22.43	22.57	22.67	22.60	22.93	22.90	22.97

Table 2. Weights of wt mice receiving 120 ppm selenomethionine in the drinking water.

While the selenium study was underway the chemotherapy regimen was being tested in XPC -/- mice, as described in the second task of the Statement of Work. As in the wildtype mice, the dosing protocol was begun with 60 mg/kg of carboplatin. After three doses of 60 mg/kg the dose was increased to 100 mg/kg. After 1 dose of 100 mg/kg the XPC -/- mice were sufficiently myelosuppressed and losing sufficient weight to stop the treatment. The results are shown in figure 2.

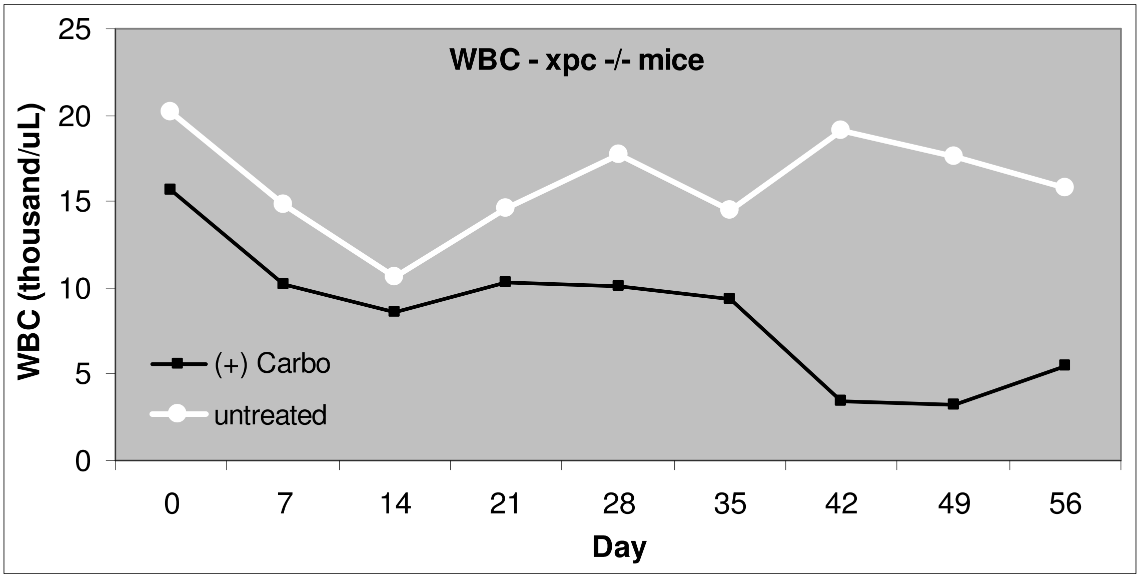

Figure 2. White blood cell counts from XPC -/- mice on a carboplatin chemotherapy regimen. Carboplatin treatment was administered at 60 mg/kg on days 9, 16, 23 and at 100 mg/kg on day 30. Figure is representative of 2 independent experiments.

The XPC -/- mice were found to be significantly more sensitive to the carboplatin treatment than the wildtype mice. While the wildtype mice were able to tolerate the full 5 doses of carboplatin therapy the XPC -/- mice suffered such significant weight loss after 4 doses that they had to be euthanized. Figure 3 compares the sensitivity of the wildtype and XPC -/- mice.

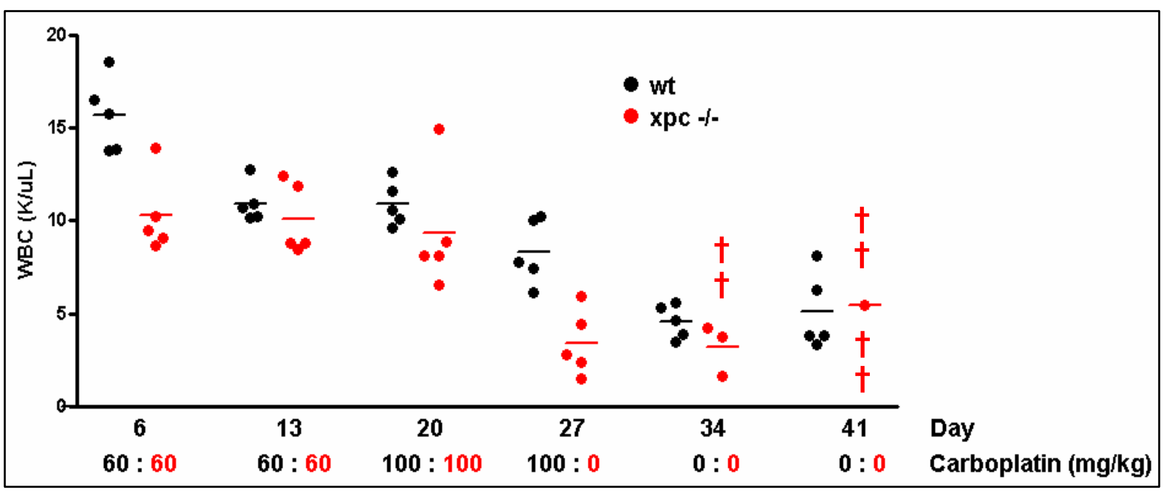

Figure 3. White blood cell counts from XPC -/- and wildtype mice on the carboplatin chemotherapy regimen. Crosses indicate animals that had to be euthanized due to excessive weight loss. Figure is representative of 2 independent experiments.

Bone marrow was harvested from the wildtype and XPC -/- mice and cultured for progenitor assays. Briefly, bone marrow was cultured in methylcellulose medium containing cytokines and colonies were counted after 10 days in culture. Bone marrow from the XPC -/- mice produced significantly fewer colonies than bone marrow from

wildtype mice indicating that the XPC -/- mice are much more sensitive to the carboplatin regiment than the wildtype mice. The results are shown in figure 4.

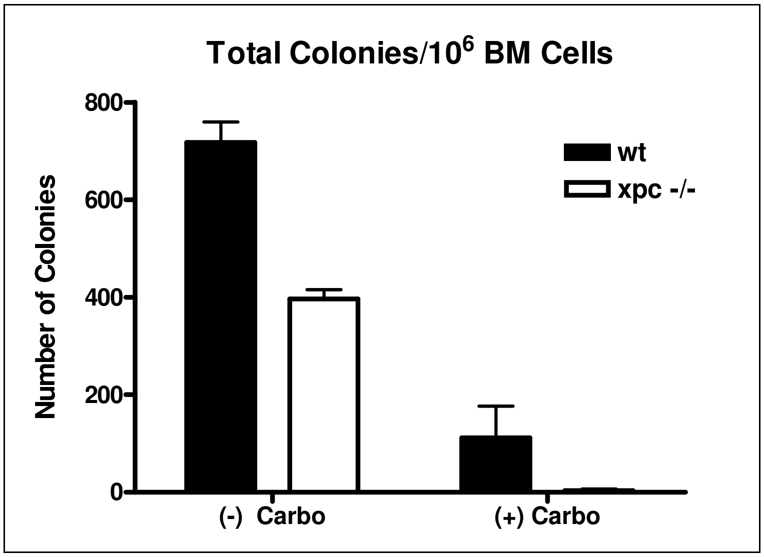

Figure 4. Total number of colonies derived from the bone marrow of wildtype and XPC -/- mice. The number of colonies is proportional to the vitality of the hematopoietic system.

KEY RESEARCH ACCOMPLISHMENTS.

- Published preliminary studies from original grant proposal
- Established dosing protocol for carboplatin chemotherapy regimen
- Found significant protection of hematopoietic system in wildtype mice given selenomethionine prior to and during carboplatin chemotherapy.
- Observed significant sensitivity of XPC -/- mice to carboplatin chemotherapy

REPORTABLE OUTCOMES.

- Manuscript published in Molecular Cancer Therapeutics "Chemotherapeutic selectivity conferred by selenium: a role for p53-dependent DNA repair." (Fischer et. al.)

CONCLUSION.

The selenium protective effects in mice on a carboplatin chemotherapy regimen must be investigated using the oral gavage method rather than supplementing the drinking water with selenium. The drinking water method would be preferable so as to not cause added stress to the animals, but it is an inadequate method of selenium incorporation. The oral gavage method has been initiated and will be incorporated into the carboplatin chemotherapy experiments already underway.

The carboplatin chemotherapy regimen developed is adequate to test the aims of the proposal. Furthermore, the carboplatin regimen revealed that the XPC -/- mice are significantly more sensitive than the wildtype mice. Progenitor assays reveal that the bone marrow in the XPC -/- mice is significantly more sensitive to the carboplatin. It is known that XPC -/- cells are more sensitive to DNA damage, but the dogma in the literature is that the contribution to cell survival is 2-3 fold. This is the first study to look at the contribution of XPC to cell survival *in vivo,* and early results appear to challenge the validity of the prevailing dogma. The results raise important points regarding the role of p53 in protecting cells from DNA damage.

REFERENCES.

Fischer JL, Mihelc EM, Pollok KE, Smith ML. Chemotherapeutic selectivity conferred by selenium: a role for p53-dependent DNA repair. Mol Cancer Ther 2007;6:355-361.

APPENDIX.

Chemotherapeutic selectivity conferred by selenium: a role for p53-dependent DNA repair

Joshua L. Fischer,[1,3] Elaine M. Mihelc,[1,3] Karen E. Pollok,[2] and Martin L. Smith[1,3]

[1]Department of Microbiology and Walther Oncology Center, Indiana University Cancer Center, and [2]Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine; and [3]Walther Cancer Institute, Indianapolis, Indiana

Abstract

Selenium in various chemical forms has been the subject of cancer chemoprevention trials, but, more recently, selenium has been used in combination with DNA-damaging chemotherapeutics. Specifically, selenium protected tissues from dose-limiting toxicity and, in fact, allowed delivery of higher chemotherapeutic doses. At the same time, selenium did not protect cancer cells. Therefore, we seek to define the genetic basis for the observed selectivity of selenium in combination chemotherapeutics. The tumor suppressor p53 is mutated in the vast majority of cancers, but is by definition wild-type in nontarget tissues such as bone marrow and gut epithelium, tissues that are often dose-limiting due to DNA damage. We used primary, low-passage mouse embryonic fibroblasts that are wild-type or null for *p53* genes to test differential effects of selenium. Seleno-L-methionine, nontoxic by itself, was used to pretreat cell cultures before exposure to UV radiation or UV-mimetic cancer chemotherapy drugs. Seleno-L-methionine pretreatment caused a DNA repair response, which protected from subsequent challenge with DNA-damaging agents. The observed DNA repair response and subsequent DNA damage protection were p53 dependent as neither was observed in p53-null cells. The data suggest that (*a*) p53 may be an important genetic determinant that distinguishes normal cells from cancer cells, and (*b*) combinatorial chemotherapeutics that act by p53-dependent mechanisms may enhance chemotherapeutic efficacy by increasing the chemotherapeutic window distinguishing cancer cells from normal cells. [Mol Cancer Ther 2007;6(1):355–61]

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Requests for reprints: Martin L. Smith, Indiana University Cancer Center, Indiana University School of Medicine, Room 155, 1044 West Walnut Street, Indianapolis, IN 46202. Phone: 317-278-0225; Fax: 317-274-7592. E-mail: marlsmit@iupui.edu

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Introduction

The majority of investigation with selenium has emphasized cancer chemoprevention, and there are a number of large prevention clinical trials, many focusing on prostate cancer (1). The potential role for selenium in cancer chemotherapeutics is an area that has shown significant promise in preclinical and small clinical trials, but this potential has been overshadowed by the prevention studies. Compelling preclinical work has shown that nude mice bearing human tumor xenografts that received daily seleno-L-methionine (SeMet) supplementation before and during chemotherapy better tolerated increasing doses of irinotecan. Dose escalation allowed elimination of previously chemoresistant tumors (2). A clinical trial using selenium supplementation during chemotherapy has been initiated based on these results (3). Furthermore, phase I trials have shown that SeMet can be administered in very high doses without significant toxicity (4, 5).

There have been relatively few clinical trials investigating the effect of selenium supplementation during cancer chemotherapy; nevertheless, the results have been positive. Forty-one patients undergoing cisplatin chemotherapy were randomized into two groups, and the group that received selenium showed significantly higher WBC counts on day 14 after initiation of chemotherapy (6). Furthermore, consumption of granulocyte colony-stimulating factor and volumes of blood transfusion were significantly less in the selenium-supplemented group. An ovarian cancer study was done with 62 women undergoing cisplatin and cyclophosphamide combination chemotherapy and half of the patients received selenium supplementation (7). The group that received selenium showed significantly reduced neutropenia as well as increased WBCs from the second to third chemotherapy cycle. The authors also report that with selenium supplementation, there seemed to be a significant decrease in all cited side effects: nausea, vomiting, hair loss, etc. It was also noted that serum and tissue selenium levels in the control group decreased during the chemotherapy regimen whereas levels in the study group increased. Neither of these studies observed any loss of chemotherapeutic efficacy in association with selenium supplementation.

Of the major types of DNA repair, nucleotide excision repair (NER) is the repair pathway responsible for removing bulky lesions. For example, 6-4 photoproducts and cyclobutane pyrimidine dimers caused by UV radiation are repaired by NER. Similarly, platinum-DNA adducts formed by platinum-containing cancer chemotherapeutics are repaired by NER (8, 9). NER is divided into two distinct pathways: global genomic repair and transcription-coupled repair. Both pathways have three

basic steps: recognition of the damaged lesion, excision of the lesion, and resynthesis. The pathways differ in the initial recognition step but use the same proteins for the subsequent steps.

The damage recognition step of NER is rate limiting. For global genomic repair, regulation of this step is controlled by p53. Cells that have defective p53, such as those from patients with Li-Fraumeni syndrome, have defective global genomic repair but retain proficient transcription-coupled repair (10–13). p53 regulates the rate-limiting step in global genomic repair through transcriptional control of the DNA damage recognition proteins xeroderma pigmentosum complement groups C (XPC) and E (XPE). It has been shown that p53 transcriptionally regulates p48/XPE/DDB2, and forced overexpression of p48/XPE/DDB2 enhances global genomic repair (14–17). Likewise, XPC mRNA and protein expression is increased in a p53- and DNA damage–dependent manner (18). It has also been shown that within minutes of UV irradiation, p48 and XPC proteins localize to the damaged sites and that p48 enhances XPC binding (15). Several studies highlight the analogous repair of UV-damaged DNA and damage caused by platinum chemotherapeutics. $XPC^{-/-}$ cells are defective in the repair of cisplatin damage, and it has been shown that XPC protein is required for cisplatin damage recognition (19, 20).

A role for selenium in DNA repair was first noticed when selenium treatment was shown to enhance host cell reactivation of a UV-damaged reporter plasmid template (21). It was later shown that selenium could only modulate DNA repair in cells with normal p53 (22). Selenium protection from DNA damage requires redox factor 1 (Ref1), which interacts with p53 and reduces key p53 cysteine residues (22, 23). The selenoprotein thioredoxin reductase is also required for p53 cysteine reduction (24). A dominant-negative Ref1 mutant blocked SeMet-induced transactivation by p53 (22). The reduced conformation of p53, promoted by SeMet, induces its transcription factor activity and the transcriptional activation of proteins responsible for recognition of DNA damage. Furthermore, the subsequent results show that SeMet elevates DNA repair and protects cells from DNA damage in the absence of cell cycle arrest or apoptosis. A potential rationale for this differential activity by p53 is likely due, in part, to posttranslational effects. It has been shown that different chemical forms of selenium have different effects on p53 phosphorylation, which alter the cellular response (25–27).

Selective modulation of NER has significant implication for patients being treated with DNA-damaging chemotherapeutic agents. The following results show that bone marrow and gut epithelium exhibited enhanced DNA repair following selenium treatment. The DNA repair activity of the cancer cells was unaffected. This effect may allow patients to receive more intense treatment without exacerbating unpleasant side effects. Experiments using matched isogenic cell lines, as well as tumors and genetically normal tissues, show that a selenium-inducible DNA repair response protects from DNA damage and is p53 dependent. Selenium treatment did not protect or increase DNA repair in p53-deficient cells.

Materials and Methods

Chemotherapeutic Drugs

Cisplatin (purchased from Sigma, St. Louis, MO) was dissolved in DMSO as a 10 mmol/L stock solution. Carboplatin was used in some experiments instead of cisplatin, and results were identical. Oxaliplatin (purchased from HandiTech, Houston, TX) was dissolved in sterile water as a 10 mmol/L stock solution. All chemotherapeutics were frozen in small aliquots and stored at −20°C. Final concentrations in tissue culture medium were as indicated. Interleukin-6 and stem cell factor were purchased from PeproTech (Rocky Hill, NJ).

Cell Lines and Treatments

Mouse embryonic fibroblasts (MEF) of wild-type and $p53^{-/-}$ genotypes were of low passage from our frozen stocks as previously described (28). MEF were from a C57/129 genetic background. Noncancer cells were IEC6 rat gut epithelial cells (American Type Culture Collection, Rockville, MD) and primary mouse bone marrow cells (C57/129). Bone marrow cells were stimulated with interleukin-6 (200 units/mL) and stem cell factor (100 ng/mL) for 24 h, then treated with SeMet (10 μmol/L) for 15 h, followed by DNA damage by cisplatin or oxaliplatin at the concentrations indicated. Cancer cell lines of human origin A253 and FaDu were from a previous study (2, 29). Both are squamous cell carcinoma of head and neck lines and carry mutant *p53* genes. FaDu carries a R248L mutant p53 allele (30), whereas A253 carries deletions in both p53 alleles (31). Xeroderma pigmentosum XPA cells defective in DNA repair served as a negative control for some experiments, as previously described (28). Cell lines were likewise treated with SeMet (10 μmol/L, 15 h) and then with DNA-damaging chemotherapeutic drugs at concentrations and durations indicated. MEF were grown in DMEM (4.5 g/L glucose) plus 10% fetal bovine serum. Other cell lines were maintained in RPMI 1640 plus 10% fetal bovine serum, except for bone marrow, which was maintained in Iscove's modified Dulbecco's medium plus 20% fetal bovine serum, interleukin-6 (200 units/mL), and stem cell factor (100 ng/mL).

Cell Survival

Cell yield was determined by thiazolyl blue assay 7 days after DNA-damaging treatments. This assay can be applied to all cell lines irrespective of their colony-forming ability, a consideration for the MEF and other primary cells, which do not form colonies. Cells were plated at ~1,000 per well in 96-well culture plates, allowed to attach for 24 h, and treated with SeMet (10 μmol/L, 15 h) and then with DNA-damaging drugs for 2 h. Drugs were removed by washing the wells in culture medium with aspiration, then medium was replaced for the 7-day duration. On day 7, 50 μL of 2 mg/mL thiazolyl blue reagent were added to each well

and plates returned to incubator for 4 h to allow formation of a blue precipitate. The amount of blue precipitate was proportional to the number of viable cells by visual inspection. Precipitates were dissolved in DMSO and quantified by a Tecan plate reader at a wavelength of 592 nm. Data were normalized to control cells that did not receive DNA damage and expressed as percent cell yield relative to untreated controls. Data were averaged from three or more independent determinations, with wells in multiples of six in each experiment. Additionally, clonogenic cell survival was determined in some data sets. Clonogenic cell survival was conducted as described (32).

Unscheduled DNA Synthesis

DNA repair synthesis or unscheduled DNA synthesis was determined as previously described (28). Cells were treated with SeMet (15 h, 10 μmol/L), then with DNA-damaging agents to induce unscheduled DNA synthesis. The prototype DNA-damaging agent was UV radiation (20 J m^{-2}, 254 nm), which served as a positive control to induce unscheduled DNA synthesis (28). XPA cells served as a negative control because they are severely defective in nucleotide excision DNA repair (NER, <1% of normal) yet they are healthy cells unless exposed to DNA damage. After UV radiation, cellular DNA was labeled with tritiated thymidine (10 μCi/mL) in tissue culture medium for 3 h. Cisplatin (100 μmol/L) or oxaliplatin (100 μmol/L) was delivered to cells for 5 h concurrent with tritiated thymidine uptake. Cells were fixed on glass slides in ethanol, then processed for autoradiography. S-phase nuclei were strongly labeled by the tritiated thymidine and were excluded from analysis. Non–S phase nuclei, primarily in the G_1 phase of the cell cycle, exhibited DNA repair synthesis (unscheduled DNA synthesis). The number of DNA repair sites per nucleus was determined, and ≥200 nuclei were assayed for each data set.

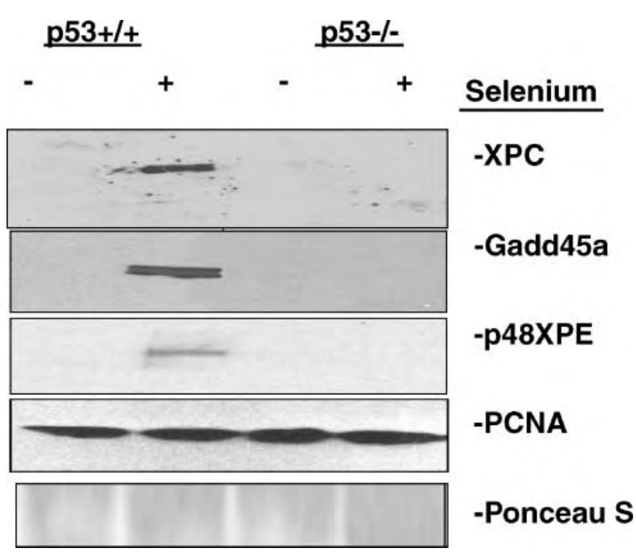

Figure 1. SeMet treatment (10 μmol/L, 15 h) caused elevated expression of p53-dependent DNA repair proteins XPC, XPE, and Gadd45a, which compose the ''DNA repair branch'' of the p53 pathway. Immunoblots were conducted with wild-type and $p53^{-/-}$ MEF. DNA repair proteins were not detected in $p53^{-/-}$ MEF. Proliferating cell nuclear antigen (*PCNA*) immunoblot and Ponceau S staining served as loading controls.

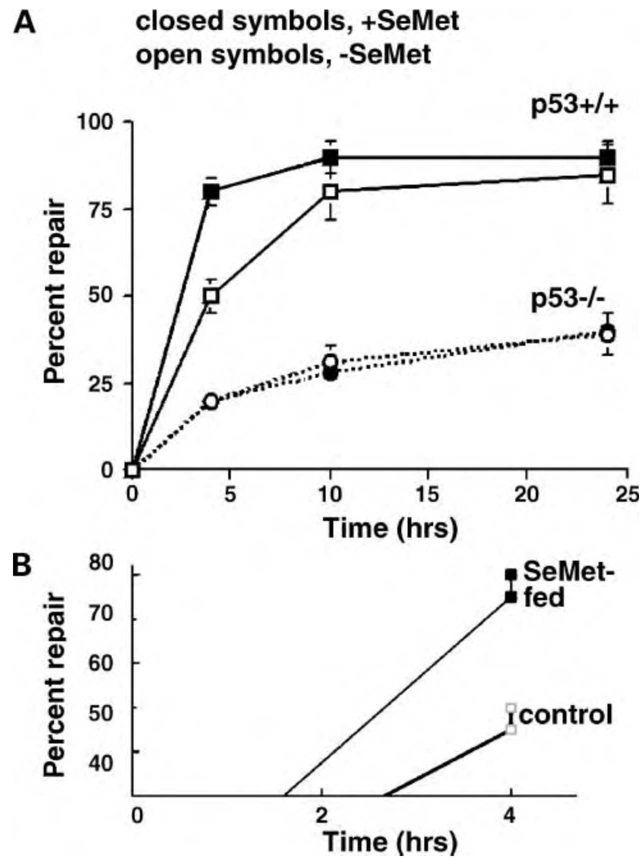

Figure 2. **A,** SeMet treatment (10 μmol/L, 15 h) increases the rate of repair of UV-induced DNA damage. MEF were treated with SeMet, then with UV radiation (20 J m^{-2}, 254 nm), and allowed indicated times for removal of UV lesions. An antibody to 6-4 photoproducts was used to assay 6-4 photoproduct removal from genomic DNA. The rate of 6-4 photoproduct removal was enhanced by selenium in wild-type MEF, but $p53^{-/-}$ MEF were unaffected. *Points,* mean of three independent determinations; *bars,* SD. $P < 0.04$, Wilcoxon rank-sum test. Note the slow rate of lesion removal in $p53^{-/-}$ MEF. **B,** *in vivo* evidence for a DNA repair response to SeMet. Feeding of mice with 200 μg/d 200 μg/d SeMet × 5 wk leads to increased DNA repair. Removal of 6-4 photoproducts was determined as in **A.**

Results

SeMet and Protein Expression

In cells treated with SeMet overnight, p53 is reduced to its transcriptionally active conformation and induces expression of NER damage recognition factors. XPC and p48XPE proteins are the main contributors to damage recognition in NER. Wild-type and $p53^{-/-}$ MEF were treated overnight with SeMet. The selenium-induced expression of damage recognition proteins is p53 dependent. Wild-type cells treated with SeMet had increased

expression of several proteins known to be involved in NER DNA damage recognition whereas $p53^{-/-}$ cells showed no change in expression of these factors (Fig. 1). Proliferating cell nuclear antigen and Ponceau S staining served as loading controls.

SeMet and Repair Rate

SeMet induces expression of damage recognition factors and has been shown to protect from DNA damage. Using an antibody to 6-4 photoproducts, a prototypical UV-inducible lesion, and cells exposed to UV radiation, the rate of repair can be assayed by monitoring the persistence of damaged lesions. Following overnight SeMet treatment, cells with wild-type p53 have fewer lesions at the indicated times (Fig. 2A). Furthermore, persistence of lesions in $p53^{-/-}$ cells is not affected. Untreated cells serve as controls. Repair rates following SeMet treatment are expressed relative to untreated controls.

To ascertain if a DNA repair response to SeMet occurs *in vivo*, mice were given 200 μg/d SeMet orally for 5 weeks. Total bone marrow cells were UV irradiated and then incubated in tissue culture for 4 h to repair. Removal of 6-4 photoproducts was determined. Repair rates by SeMet feeding are shown relative to control mice (Fig. 2B).

SeMet and Chemotherapy

Selenomethionine protects wild-type MEF from UV radiation or cisplatin (Fig. 3). $p53^{-/-}$ MEF were not protected. Cells were pretreated with 10 μmol/L selenomethionine for 15 h before DNA-damaging treatments. Cell survival was determined after 7 days by thiazolyl blue assay. Data of cell yield after 7 days are expressed relative to controls not treated with selenomethionine and controls not treated with DNA-damaging agents. The results shown for UV radiation are similar to those previously published (22). The similar results for UV and cisplatin treatment reiterate the requirement for p53-mediated NER for both types of damage. Cisplatin concentrations were as indicated. The implication is that p53 status is a molecular determinant that mediates DNA repair by selenium. The following experiments address this possibility.

SeMet and DNA Repair

The above findings show that SeMet treatment induces expression of NER damage recognition factors and elevates the rate of repair. Furthermore, SeMet protected wild-type, but not $p53^{-/-}$, cells from DNA damage. The unscheduled DNA synthesis assay was used to assay DNA repair *in vitro*. The method is illustrated in Fig. 4A. Isogenic wild-type and p53-deficient MEF (Fig. 4B) were treated with selenomethionine and various DNA-damaging agents and then unscheduled DNA synthesis was evaluated. Additionally, the effect of selenomethionine on DNA repair was evaluated in primary rat gut epithelial cells (IEC6), primary murine bone marrow, and two of the human squamous cell carcinoma of the head and neck (A253 and FaDu) cell lines used for xenografts in Cao et al.'s (2) study (Fig. 4C). Cells were treated with a variety of DNA-damaging agents: UV, cisplatin, or oxaliplatin. Wild-type MEF, rat gut epithelial cells, and murine bone marrow with genetically normal p53 show a significant increase in unscheduled DNA synthesis when treated with selenomethionine before DNA damage $P < 0.02$ (*t* test). Cells lacking functional p53 [A253 (p53 mut), FaDu ($p53^{-/-}$), and $p53^{-/-}$ MEF] were unresponsive to selenomethionine and showed no increase in unscheduled DNA synthesis.

SeMet Metabolites

Besides being used as seleno-amino acids for selenoprotein synthesis, low molecular weight metabolites of selenium compounds can mediate some biological responses. We used methyl selenenic acid as a representative SeMet metabolite. Although methyl selenenic acid showed some evidence for a DNA repair response at <1 μmol/L concentration (27), apoptosis predominated at methyl selenenic acid concentrations >1 μmol/L (Fig. 5). The DNA repair and protective effect of SeMet are therefore not likely due to low molecular weight metabolites.

Discussion

Clinical trials have shown that selenium supplementation during chemotherapy may partially alleviate the

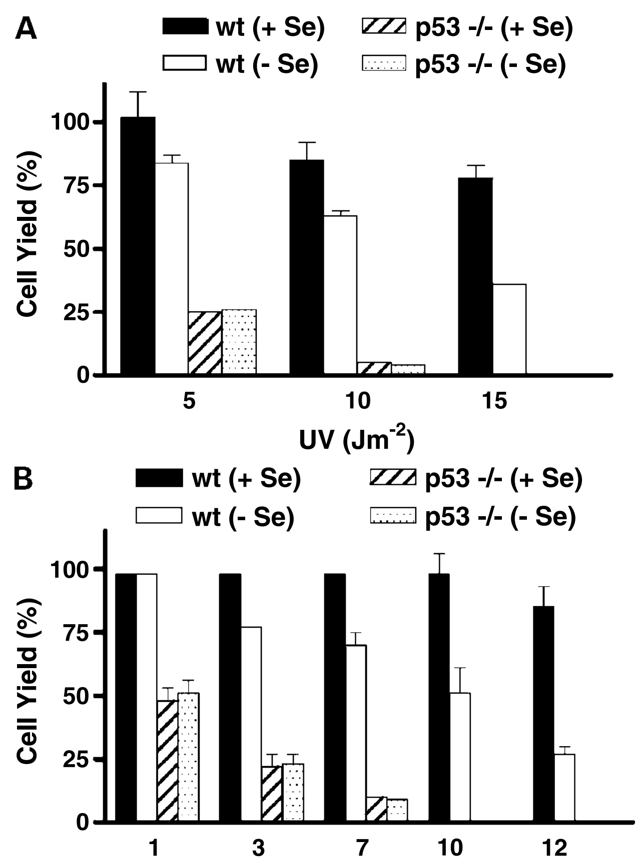

Figure 3. SeMet treatment (10 μmol/L, 15 h) promotes cell survival in wild-type, but not $p53^{-/-}$, MEF. MEF were treated with SeMet, then with 254-nm UV radiation (**A**) or cisplatin (**B**). Cell survival was determined after 7 d by thiazolyl blue assay. *Columns,* mean of three independent determinations; *bars,* SD. $P < 0.04$, *t* test.

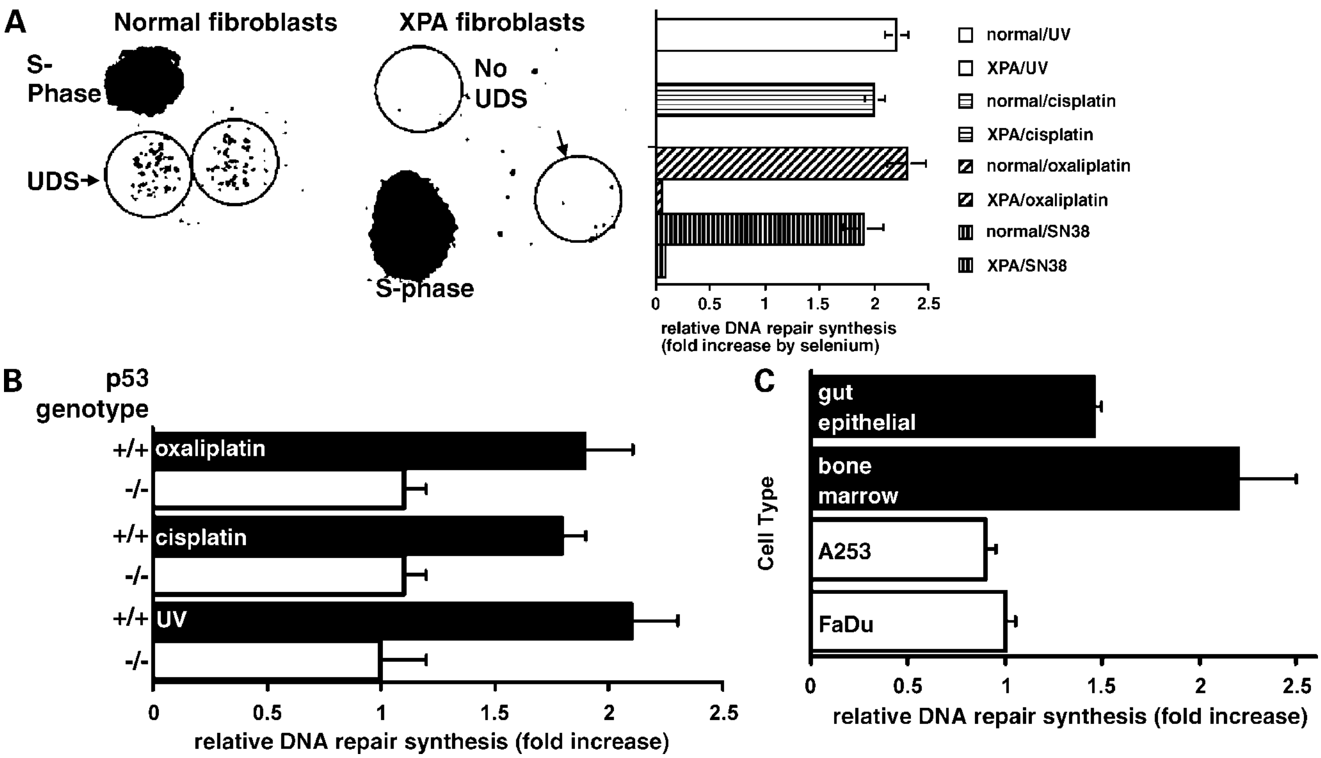

Figure 4. SeMet enhanced global genomic DNA repair as unscheduled DNA synthesis in wild-type, but not $p53^{-/-}$, MEF. **A,** illustration of methods and controls. Controls (normal human fibroblasts and DNA repair–defective XPA fibroblasts) were UV irradiated (20 J m^{-2}, 254 nm) and incubated in the presence of tritiated thymidine for 3 h, during which time the tritium label was incorporated into NER repair patches. Slides were processed for autoradiography. S-phase nuclei were excluded from analysis. By definition, unscheduled DNA synthesis (*UDS*; or repair synthesis) is confined to G_1 and G_2 nuclei. The number of tritium grains per nucleus is a direct measure of sites of repair synthesis. Cells not treated with DNA-damaging agents showed little or no unscheduled DNA synthesis (28). **B,** MEF treated with UV radiation (20 J m^{-2}, 254 nm), cisplatin (50 μmol/L), or oxaliplatin (1 μmol/L) for 4 h concurrent with tritiated thymidine labeling. SeMet was added to the medium 15 h before DNA-damaging treatments. Shown is relative repair synthesis (SeMet treated divided by SeMet untreated for each respective sample) in G_1 nuclei; *bars,* SD. At least 200 nuclei were determined per data point. SeMet induced NER in wild-type MEF ($P < 0.01$, *t* test). SeMet did not significantly induce NER in $p53^{-/-}$ MEF. **C,** SeMet induced NER in normal mouse bone marrow and in primary rat gut epithelial cells ($P < 0.01$, *t* test) but did not significantly induce NER in p53-mutant cancer cell lines A253 and FaDu.

dose-limiting and poor quality-of-life side effects. In two studies, selenium supplementation significantly reduced myelotoxicity, and in one study, selenium reduced other side effects attributed to the toxicity to rapidly proliferating nontarget tissues (6, 7, 33). The findings herein show that selenium supplementation elevates expression of proteins responsible for recognition of DNA damage. The increased expression of recognition factors is concomitant with an increase in the rate of DNA repair and overall DNA repair synthesis. However, all of these selenium-inducible observations are absent in a p53-null background. That is, selenium did not induce expression of key NER recognition factors or alter the rate or overall level of DNA repair in the p53-null cells or tumor cell lines tested. The conclusion is that selenium selectively protects genetically normal cells from DNA-damaging chemotherapeutics, while simultaneously offering no detectable protection to cells either completely lacking p53 or possessing only mutant p53. This is important considering that p53 is the most widespread genetic alteration in human cancer, with as many as 70% of tumors having a mutant p53 phenotype. One caveat is that some cancers with wild-type p53 may not be ideally suited for selenium therapies.

The results suggest a potential mechanism for selenium-inducible protection from chemotherapy in the clinical trials highlighted above and in the context of chemoprevention. In the nontarget tissues, an increase in the basal levels of NER damage recognition factors following selenium supplementation promotes an increase in the basal rate of NER, which can better tolerate the additional damage from chemotherapy. The elevated DNA repair synthesis in cells from nontarget tissues in this report, combined with the data from an earlier study showing selenium enhancing cure rates of xenograft tumors in nude mice (2), supports the proposed mechanism of selectivity.

The notion that p53 is an important marker for differentiating tumor cells from normal cells is not new. It is important to note, however, that a safe, reliable therapy that takes advantage of this widely known fact remains to be identified. The widespread p53 mutations in

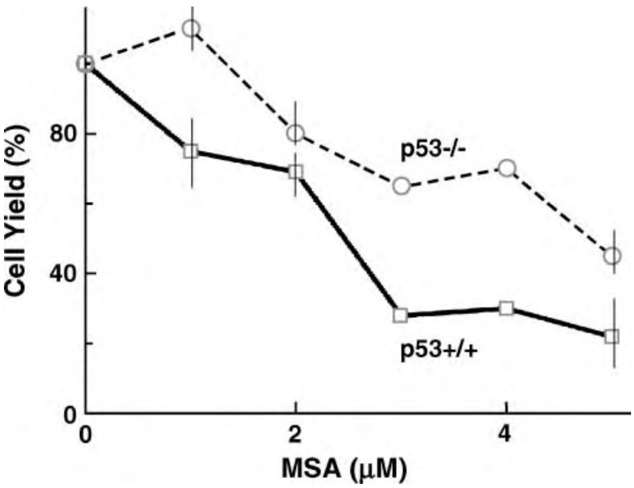

Figure 5. The selenium metabolite methyl selenenic acid did not induce a protective response in MEF. Rather, p53-mediated apoptosis predominated at methyl selenenic acid (*MSA*) concentrations >1 μmol/L. p53$^{+/+}$ and p53$^{-/-}$ MEF were treated with indicated concentrations of methyl selenenic acid for 4 h. Cell survival was determined after 5 d in culture. p53$^{+/+}$ MEF were preferentially sensitive to methyl selenenic acid ($P < 0.02$, Wilcoxon rank-sum test).

human cancer should be a benchmark for developing novel therapies. However, the inherent heterogeneity of tumors and their unpredictable responses to therapeutic strategies require extensive testing of tumor tissue. Whereas cells with altered p53 should be more sensitive to agents whose damage is repaired by the p53-regulated NER pathway, tumor cells have acquired other growth advantages, which may abrogate this potential weakness (34). A typical proposal for improving chemotherapeutic efficacy attempts to sensitize tumor cells by targeting their greatest defenses (e.g., apoptotic, cell cycle, and DNA repair targets). A strategy that protects normal cells instead is perhaps more reliable. Selenium supplementation has recently been shown in clinical trials to be nontoxic at very high doses (4). In fact, ongoing trials are attempting to reach levels of at least 15 μmol/L, which shows that the concentrations used in the present study are physiologically relevant (5). The results of this study present a safe potential method of improving chemotherapeutic selectivity that focuses on the genetically normal, nontarget tissues, which may be a more promising foundation for novel therapeutic strategies.

In the United States, serum selenium concentrations of 1 μmol/L are fairly common (1). At 1 μmol/L concentration, both seleno-amino acids exemplified by SeMet and metabolic by-products of SeMet exemplified by methyl selenenic acid may contribute to DNA repair (27). At concentrations exceeding 1 μmol/L, such as in this study, methyl selenenic acid induced apoptosis, which would mask any DNA repair response (Fig. 5). Therefore, it is likely that DNA repair and DNA damage protection observed *in vitro* (refs. 21, 22, and this study) and *in vivo* (Fig. 2B) at selenium concentrations in the 15 μmol/L range are due to selenoproteins (e.g., thioredoxin reductase). Note, however, that the apoptotic response evoked by methyl selenenic acid also involves p53, as p53-wild-type MEF were preferentially sensitive to methyl selenenic acid (Fig. 5). DNA repair or apoptotic responses would each be important in chemotherapy, albeit mediated by different selenium chemical forms.

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